Antimicrobial, Antioxidative and Antidiabetic Analysis of Crude Ethanolic and Hydro-ethanolic Extracts of *Acalypha indica*

A Synopsis

Submitted in

partial fulfillment for the degree

of

Doctor of Philosophy

(Biotechnology)

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January, 2017

Introduction

Diabetes Mellitus (DM) is widely recognized as one of the leading causes of death and disability worldwide. Diabetes is a group of metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both and it also results in the aberration of carbohydrate, fat and protein metabolism. Diabetes is a disease of increased thirst, urination, hunger, fatigue, weight loss and blurred vision. The vast majority of cases of diabetes fall into two broad categories. First one is Insulin Dependent Diabetes Mellitus (IDDM) and the second one is Non-Insulin dependent Diabetes Mellitus (NIDDM). The first one is Type 1 diabetes (IDDM) the cause is an absolute deficiency of insulin secretion. Symptoms of type 1 diabetes can start quickly, in a matter of weeks. In Type 2 diabetes (NIDDM) the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. Symptoms of type 2 diabetes often develop slowly—over the course of several years—and can be so mild that you might not even notice them. Many people with type 2 diabetes have no symptoms. Some people do not find out they have the disease until they have diabetes-related health problems, such as blurred vision or heart trouble.

In DM, elevated level of Post Prandial Hyperglycemia (PPHG) is a major risk factor and its reduction can manage DM. Therapeutic approach of anti-diabetic activity is to minimize the production of gastrointestinal glucose and inhibition of carbohydrate digesting α-amylase and α-glucosidase enzymes to control PPHG associated with diabetes. PPHG is primarily due to first phase insulin secretion. α- glucosidase inhibitors delay glucose absorption at the intestine level and thereby prevent sudden surge of glucose after a meal (Garg *et al*.2014).

Human body is continuously exposed to different types of agents that results in the production of reactive species called as free radicals (ROS)(Asmat *et al.* 2016).Reactive Oxygen Species include charged species and uncharged species such as superoxide and the hydroxyl radical, and uncharged species such as hydrogen peroxide and singlet oxygen for the latter. ROS generated by high glucose is causally linked to elevated glucose and other metabolic abnormalities important to the development of diabetic complications. There are multiple

sources of reactive oxygen species (ROS) production in diabetes including those of mitochondrial and non-mitochondrial origins; ROS accelerates the four important molecular mechanisms involved in hyperglycemia-induced oxidative tissue damage. These four pathways are activation of protein kinase C (PKC), increased hexosamine pathway flux, increased advanced glycation end-product (AGE), and increased polyol pathway flux (Rolo *et al*. 2006).

Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes increased lipid peroxidation and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus. (Khan *et al*. 2015)Cells have evolved highly complex enzymatic and non-enzymatic antioxidant systems, which work synergistically, and in combination with each other, to protect the body against free radical-induced damage. There are several lines of evidence to suggest that antioxidant defenses may be lower in diabetes. These include reports of reduced plasma/serum total antioxidant status or free radical scavenging activity and increased plasma oxidisability in type 2 diabetics, together with reduced levels of specific antioxidants such as ascorbic acid and vitamin E. In addition, the activities of the antioxidant enzymes catalase, superoxide dismutase, and glutathione peroxidase have been described as reduced in diabetic patient.(Laight et al. 2000).Antioxidant can be classified as enzymatic and non-enzymatic. Antioxidant enzyme can be further classified as superoxide dismutase (It is an enzyme with a generalized presence in the body which catalyzes the dismutation of superoxide), Gluthathione (It is defensive in natureand helps to neutralize hydrogen peroxide produced inside the cell). Non- enzymatic antioxidant include vitamin C, also called ascorbic acid, glutathione, phenolic compounds, vitamin A, vitamin E, β-carotene etc.

An antimicrobial activity is one of the only agent that used to kill microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms, they act primarily against other micro-organisms which are the main cause of the disease.

In 2000, the (WHO) recorded a total of 171 million people for all age groups worldwide (2.8% of the global population) who have diabetes, and the numbers are expected to rise to 366 million (4.4% of the global population) by 2030. Alogliptin is an anti-diabetic drug, prescribed for type 2 DM. The balance between the rate of free radical generation and elimination is important. Excess free radical generation can be harmful; however, if there is a significant increase in radical generation, or a decrease in radical elimination from the cell, oxidative stress ensues.

Antibiotics are used against bacteria and anti fungals are used against fungi. Metformin, Sulfonylureas, Biguanides, Meglinitides, Thiazolidinediones, (Mohammed *et al*.2013). α-glucosidase inhibitors are most widely prescribed oral anti-diabetic drug used for the treatment of non-insulin-dependent Diabetes Mellitus (NIDDM) patients (Scheen A.J *et al.*1993).Acetohexamideis a first-generation sulfonylurea agent, prescribed for the chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of various organs such as eyes, kidneys, nerves, heart, and blood vesselsAlogliptin and Metformincontains Dipeptidyl-Peptidase-4 (DPP-4) inhibitor and a Biguanide, prescribed for type 2 DM along with diet and exercise. But since ancient times, plants have been an exemplary source of medicine. Medicinal plants have the advantage of having no or minimum side effects when used as medicinal plants compared to imported synthetic alternatives.

Studies previously conducted reveals that isolation and identification of bioactive compounds present in a crude extract sample serve as a building block for the development of new types of therapies with new the treatment of the mechanisms of action with a stronger species Several human diseases (Lee *et al*., 2000). .

Plant extracts are generally produced as a combination of different types of bioactive compounds or phytochemicals of different polarity, their separation still remains a major challenge for the process of identification and characterization of bioactive compounds. It is a common practice for the isolation of these bioactive compounds as a number of different separation techniques such as TLC, column chromatography, flash chromatography, Sephadex chromatography and HPLC should be used to obtain pure compounds.

Acalypha indica(Linn) consists of dried herb of *Acalypha indica* (Linn). belonging to family Euphorbiaceae. Within the Euphorbiaceae family. *Acalypha indica* is one of the biggest and most diverse, within early 450 species. Two thirds of its species are found in America, 19 of them in Venezuala, they are mainly used as

ornamental, but with the rising of traditional medicine, these species are givenmedical properties painkillers, laxatives, anti-inflammatory and many others *Acalypha indica(*Linn).Occurs in Nigeria and from Sudaneast to Somailia and South through DR Congo and East Africa to Southern Africa including South Africa. It is also widespread in the Indian Ocean islands and occurs furthermore in India, South-EastAsia and Oceania. It was introduced into the warmer parts of the New World. It is found throughout the plains of India as weed in gardens, in waste lands and along the roadsides. It is found in the hills of Orissa at altitudes upto 210 m. A part of the supply also comes from West Bengal, Andhra Pradesh, Tamil Nadu, and Kerala. (Masih *et al.*2011).

Objectives

1. To evaluate the phytochemical investigation of ethanolic and hydro-ethonlic extracts of *Acalypha indica* (Linn)

2. To screening the anti-microbial activity of ethanolic and hydroethanolic extracts of *Acalypha indica* (Linn)

3. To evaluate the *in-vitro* anti-oxidative potential of ethanolic and hydroethanolic extracts of *Acalypha indica* (Linn)

4. To study the *in-vitro* anti-diabetic potential of ethanol and hydroethanolic extracts of *Acalypha indica* (Linn)

5. To isolation, purification and identification of active principle compounds from the extract showing the antihyperglycemic and antioxidative potential

Review of Literature

Abere *et al.* (2007) explains *in-vitro* antimicrobial activity of the extract of Mitracarpus scaber leaves. They formulated the crude extract of the leaves of Mitracarpus scarber ―Zucc‖ as a syrup.The antimicrobial activity of the formulation was assessed using agar plates and concentrations of the extract varying between 25mg/ml and 300mg/ml to determine the minimum inhibitory concentration at 37^0C against bacterial and fungal organisms namely, *Escherichia coli*., *Pseudomonas aeruginosa*. The growth of E. coli was inhibited by the formulation at a minimum inhibitory concentration of 75mg/ml. Pseudomonas aeruginosa resist all the concentrations of the formulation used. The presence of sucrose in the formulation rendered the formulation pleasant to taste.The extract from the leaves of Mitracarpus scarber ―Zucc‖ can be formulated into a pleasantly tasting oral dosage form despite its bitter taste.

Deore *et al*. (2008) investigates *in-vitro* antimicrobial and antioxidant studies on *Enicostemma axillare* (Lam.) *Raynal.* Leaves. *Enicostemma axillare* (Lam.) *Raynal. syn. E. littorale Blume* (Family ¾ Gentianaceae).It is a perennial herb found throughout the greater part of India. In present *in-vitro* antimicrobial activity (Well diffusion method) of aqueous, hydro alcoholic, methanolic, chloroform and ethyl acetate extract of leaves of this plant has been evaluated. Six bacterial species and two fungal strains used for study are: *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella sonni*, *Aspergillus niger* and *Candida albicans*. It was observed that chloroform, ethyl acetate and hydroalcoholic extract showed prominent antimicrobial activity against all microorganisms. *In-vitro* antioxidant activity of each extract except ethyl acetate and the possible mechanism involved was investigated by using two different model covering nitric oxide and DPPH method. The result indicated efficacy of extracts for antioxidant activity in following sequence: methanol>hydro alcoholic>aqueous>chloroform.

Nile *et al.* (2009) investigates antioxidant activity and flavonoid derivatives of *Plumbagozeylanica*. The plant roots extract reveled significant antioxidant activity as compared to standard flavonoid (quercetin). The antioxidant activity by DPPH is 96µg/ml and by NBT is 4.6µg/ml which greater than that of standard

(Quercetin) 45 µg/ml by DPPH and 10µg/ml by NBT assay. The phytochemical investigation showed presence of flavonoids, tannins and saponins. The total phenolic and total flavonoid content was found to be 260±48.0 and 45.5±5.2 mg of GAE/g and CE/g respectively.

Reddy *et al*. (2010) evaluates the *in-vitro* antioxidant and *in-vitro* antidiabetic activity of methanolic extract of leaves of *Asystasia gangetica* in various models. Besides, total phenolic was tested using Folin Ciocalteau reagent. α-glucosidase and α-amylase enzyme inhibition may be the possible mechanism for the plant to exert antidiabetic activity and considered as a potential candidate for the management of type-II diabetes mellitus. The *in-vitro* studies clearly indicate that the methanol extract of leaves of *Asystasia gangetica* has significant *in-vitro* antioxidant and α-glucosidase and α-amylase enzymes inhibitory activity.

Hawaze *et al*. (2012) explains *in- vitro* antimicrobial activity and phytochemical screening of Clematis Species. *Clematis longicauda steud* ex A. Rich. and *Clematis burgensis Engl*. are used in Southwestern Ethiopia to treat otorrhoea and eczema. Antimicrobial activity and MIC of crude extracts were determined by disk diffusion and broth dilution. Phytochemical screening was performed on the extracts. The methanol and petroleum ether extracts of both plants showed antibacterial and antifungal activity. Sensitivity of reference strains was concentration dependent. Methanol and petroleum ether extracts of *C. burgensis* leaves exerted greater inhibitory effects than *C. longicauda* extracts whereas aqueous extracts of both plants were inactive. The MIC study revealed a concentration of 0.78 mg/ml on bacteria and 3.125 mg/ml on fungi for methanol extract and 1.56 mg/ml on both fungi and bacteria for petroleum ether extract. Phytochemical screening results indicated the presence of proteins, fixed oils, carbohydrates, tannins, saponins, flavonoids, and steroids. Preliminary chromatographic investigation showed fluorescing spots with Rf values that ranged from 0.05 to 0.96 for phenolic compounds and saponins. As the study is one of the first reports on the two indigenous species of Clematis; isolation, purification and characterization of the different.

Savitha *et al*. (2013) studied the antibacterial effect of leaves, stem and root of *Acalypha indica* L. It was evaluated on bacterial strains like *Staphylococcus aureus* and *Salmonella typhi.* The solvent used for the

extraction of plants were water and methanol. The *in-vitro* antibacterial activity was performed by agar cup method. The most suspectible bacteria was *Staphylococcus aureus*. The significant antibacterial activity of active extract was compared with the standard antibiotic streptomycin.

Poojari *et al.* (2014) investigates the preliminary screening of phytochemicals and evaluation of *in-vitro* antidiabetic efficacy of *Physalis angulata* fruit extracts.The study reveals the presence of phytochemicals such as alkalodis, saponins, phenols etc. Among the tested extracts of fruit methanol has been successfully inhibited both of the enzymes *in-vitro*. The highest inhibition percentage 97.23 and 96.53 noticed against alpha amylase and alpha glucosidase at 100 µg/ml respectively. The results are comparable with the percentage 95.16% recorded with reference drug acarbose at 10 µg/ml.

Hossain *et al*. (2015) investigates *in-vitro* antioxidant activity, total phenolic content and concentration of flavonoids of four different extracts of *Adina cordifolia* (Roxb.) were determined using spectrophotometric methods. Antioxidant activity of extracts was expressed as percentage of DPPH radicals' inhibition and IC_{50} values (µg/ml). IC_{50} values ranged from 20.39 to 38.96 µg/ml. The total phenolic content ranged from 17.48 to 20.83 mg/g of dry weight of extract, expressed as gallic acid equivalents. The total flavonoid concentrations varied from 17.49 to 22.48 mg/g, expressed as quercetin equivalents. The significant linear correlation was confirmed between the values for the total phenolic content and antioxidant activity of plant extracts. *Adina cordifolia* (Roxb.) can be regarded as promising candidates for natural plant sources of antioxidants with high value.

Haleema *et al*. (2016) aim was to analyze the antioxidant content and *in-vitro* antioxidant activity of popped *Euryale ferox* seeds. Popped seeds were found to contain low quantities of total phenolics (52.7 mg/100g gallic acid equivalents) and flavonoids (1.25 mg/100g rutin equivalents). The ethanol and 80% methanol extracts showed 1,1 Diphenyl-2- picryl -hydrazyl (DPPH) radical scavenging activity in a dose dependant manner with half maximal inhibitory concentration (IC50) values of 109.3µg/ml, 91µg/ml respectively. Ferric reducing ability of 80% methanol and ethanol extracts were lower than butylated hydroxy toluene used as a standard and

aqueous extract had the least activity. These results suggest that popped seed extracts are relatively lower in antioxidant content and *in-vitro* antioxidant activity in comparison with reported values of extracts from raw seeds and seed shell which can be the outcome of high temperature applied during the processing of seeds.

Methodology

Procurement of plant material

The leaves, flowers, pods and stem of *Acalypha indica* were procured from Cazri Institute, Jodhpur, Rajasthan, India. Identification of the plant was done through available literature and authenticated by Dr. Swaati Sharma, Assistant Professor Department of Agriculture, Food and Biotechnology, Jayoti Vidyapeeth Women's University Jaipur (Rajasthan).

- ## Preparation of hydro-ethanolic crude extracts by Direct Soxhlet method

The plant parts have been washed thoroughly with tap water and are subjected to air drying in the shade for about 2-3 weeks. The dried parts are further reduced in their size to form coarse powder which is sieved out through Sieve No. 80 and stored in airtight containers in refrigerator. 100 grams of ground coarse powder of each plant part is packed in a filter paper thimble becomes clear concentrated to dryness. This is then extracted in 1 Liter of hydro-ethanol (50%- ethanol) by hot uninterrupted percolation using soxhlet apparatus and further refluxed for 18/24 hrs at 70°C. The liquid extracts collected are evaporated in a rotary evaporator. The reduced extracts are then collected in glass petri-plates and allowed to evaporate and form semi-solid masses. These hydro-ethanolic extracts are then labeled and preserved in desiccators with $CaCl_2$. (Garg *et al*., 2014)

Plant Part	Name of the Ethanolic and Hydro-ethanolic extract
*Acalypha indica*Leaves	AIEL and AIHEL
*Acalypha indica*Flower	AIEF and AIHEF
*Acalypha indica*Pod	AIEP and AIHEP
*Acalypha indica*Stem	AIEB and AIHES

Hydro-ethanolic extracts of various parts of *Acalypha indica*

1. **Phytochemical Screening**

Qualitative tests will be undertaken on the hydro-ethanolic extracts of aerial parts of *Acalypha indica*using standard protocols for the identification of phytochemicals (Harborne, 1973; Trease and Evans, 1989; Safowra, 1993)

Test for Phlobatanins, triterpenes, cardiacglycoside, alkaloids, fatty Acids, flavonoids, glycosides, phenols, resins, saponins, steroids, tannins, terpenoids.

2. **Antimicrobial activity**

Microrganisms used: Standardised strains of bacteria and fungi.(Salvamohan *et al*.2012)

Assay of antimicrobial activity using Disc Diffusion method.

Antimicrobial activity of commercially available antibiotics.

3. ***In- vitro* anti-oxidative activity**

- Free radical scavenging potential

ABTS (2,2'-azinobis[3ethylbenzthiazoline]-6-sulfonic acid) free radical scavenging activity

(Roberta *et al*.1999)

DPPH (Diphenyl picryl hydrazyl) activity (Blois *et al*, 1958)

Metal ion scavenging activity (Decker and Barbara, 1990)

- *In- vitro* tests for Enzymatic Antioxidants

Superoxide Dismutase (SOD)activity (Beauchamp and Fedovich, 1976)

Catalase (CAT) activity (Chance and Maehly, 1995)

Glutathione Peroxidase (gpx) activity (Starlin and Gopalakrishnan, 2013)

- *In-Vitro* Assay of Non -Enzymatic Antioxidants DPPH

Radical Scavenging Assay (Rahmatullah *et al*.2009)

The radical scavenging activity has been then estimated by calculating the inhibition percentage (I) as follows. I= (Abs control – Abs sample) / Abs control X 100

α- Glucosidase assay (Krishna veni *et al*. 2001)

Metal Chelating Activity (Minzhil *et al*.1989)

calculated relative to the control using formula.

Chelating effect = (Abs control – Abs sample) / Abs control

Hydroxyl Radical Scavenging Assay (Anjaneyulu *et al*.1989)

Inhibition percent (I) of deoxyribose degradation will be calculated using the formula.

I= (Abs control – Abs sample) / Abs control. Ascorbic acid has been used as a positive control.

4. ***In- vitro* anti-diabetic potential**

The *in-vitro* anti-diabetic activity of various extracts prepared will be carried out by:

α- amylase assay (Miller, 1959)

The results will be expressed as inhibition % calculated using the

formula: I= (Abs control – Abs sample) / Abs control x 100

α- glucosidase assay (Krishna Veni *et al. 2011)*

5. Isolation, purification and identification of active principle compounds

Isolation and purification was performed by Thin Layer Chromatography (TLC) and Column Chromatography were performed for separating the principle components from the best plant part for (antimicrobial), (antidiabetic and antioxidative) activity and the isolated compound was characterized by HPLC, FTIR, NMR and GC-MS techniques (Sharma and Garg, 2011).

- **Calculation of IC_{50}**

Anti-radical and anti-diabetic activity of testing compounds is worked out by calculating IC_{50} (concentration of a compound inhibiting the activity of a test solution by 50%) using MS Excel.

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